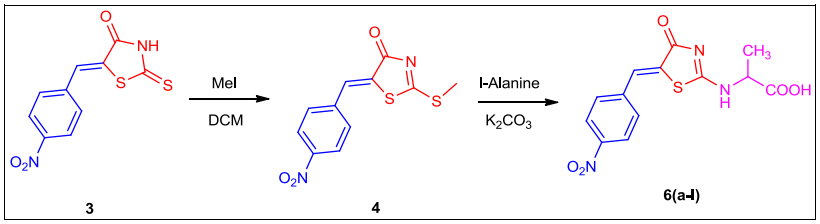
A Facial Synthesis and Anticancer Activity of (*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-substituted Acid

Dattatraya N. Pansare,[a]* Rohini N. Shelke,[b] and Devanand B. Shinde[c]

[a]Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431 004, MS, India
[b]Department of Chemistry, Deogiri College, Station Road, Aurangabad 431 005, MS, India
[c]Shivaji University, Vidyanagar, Kolhapur 416 004, MS, India
*E-mail: dnpansare74@gmail.com

Received February 9, 2017
DOI 10.1002/jhet.2919
Published online 00 Month 2017 in Wiley Online Library (wileyonlinelibrary.com).



In order to explore the anticancer and antimicrobial activity associated with the thiazole framework, we synthesized the new series (*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-substituted acid derivatives **6a**–**l**. All the synthesized compounds were evaluated for anticancer and antimicrobial activity in vitro. Among these, the compounds **6a**, **6b, 6c**, **6e**, **6f**, **6g**, **6h**, **6i**, **6j**, and **6k** showed highest antibacterial and antifungal activity. The compound **6a** exhibited significant antibacterial activity against *Bacillussubtilis*, whereas compound **6j** displays significant antifungal activity against fungal strains, that is, *A. oryzae*. The in vitro anticancer studies revealed that **6e**, **6g**, **6h**, **6k**, and **6l** are the most active compounds against MCF-7 and BT-474 human breast cancer cell lines, which can be regarded as the promising drug candidate for development of anticancer drugs.

J. Heterocyclic Chem., **00,** 00 (2017).

INTRODUCTION

The resistance of pathogenic bacteria to available antibiotics is quickly becoming a major problem in the community-based and hospital-based healthcare settings. The search for novel agents to combat resistant bacteria has become one of the most important areas of antibacterial research today [1]. Since the last five decades, a very rapid progress has been made in the area of cancer cell biology, although most cancer treatments are still multimodal, involving chemotherapy and radiotherapy with chemotherapy remains the most significant pharmacological approximation to cancer treatment [2]. The cancer is the second leading cause of death in the world after cardiovascular diseases, and it is projected to beginning the primary cause of death there within the coming year [3,4]. The breast cancer may be one of the oldest known forms of cancerous tumors in humans. Worldwide, breast cancer is the most common cancer in women, after skin cancer, representing 16% of all female cancers [5]. The heterocyclic chemistry is one of the great importance to the medicinal chemists because the steady growth of interest in heterocyclic compounds is connected with their therapeutic activity. Further, the compounds containing the 2-thioxothiazolidin-4-one (rhodanine) scaffold has been gaining prominence in recent years, because its derivatives are known to possess wide spectrum of pharmacological activities, such as antimicrobial [6–10], antidiabetic [11], anticancer [12–14], antiviral [15,16], antifungal [17], anti-dengue [18], anti-tuberculosis [19,20], and anti-HIV [21]. The identification of novel structure that can be potentially useful in designing new potent selective and less toxic anticancer agent is still a major challenge to medicinal chemistry researchers [22,23]. Recently, it was reported that substituted thiazolidinone inhibit the *MurB* enzyme, which is an integral component in bacterial peptidoglycan biosynthesis, at the low micromolar level (Fig. 1) [24,25].

In view of the aforementioned considerations, in continuation of our previous work on triazoles, pyrimidine, thiazoles, and thiazolidinones of pharmaceutical interest [26,27], we report here on the synthesis, characterization, anticancer, and antimicrobial evaluation of new synthesis of (*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-substituted acid (**6a**–**l**).

RESULTS AND DISCUSSION

Chemistry. In view of the facts mentioned previously, rhodanine derivatives were synthesized (**3**, **4** and **6a**–**l**), characterized by different spectral analytical techniques and screened for their antimicrobial activity against six bacteria, *Bacillus subtilis* (NCIM-2063), *Staphylococcus aureus* (NCIM-2901), *Escherichia coli* (NCIM-2256), *Enterococcus faecalis* (NCIM-5443), *Pseudomonas*

© 2017 Wiley Periodicals, Inc.

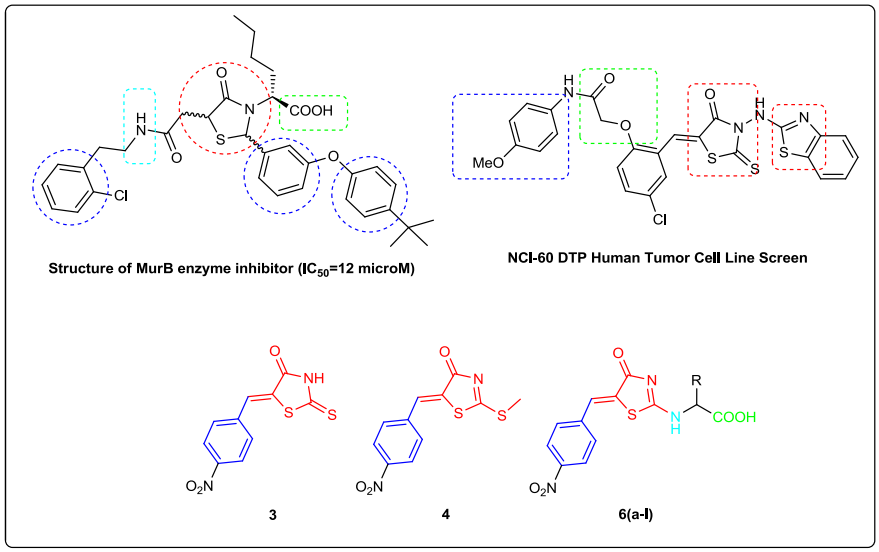


Figure 1. Previously reported *MurB* enzyme inhibitor, anticancer active, and synthesized compounds. [Color figure can be viewed at wileyonlinelibrary.com]

aeruginosa (NCIM-2037), and *Salmonella typhimurium* (NCIM-2501) and six fungal strains: *Aspergillus oryzae* (NCIM-570), *Penicillium chrysogenum* (NCIM-707), *Fusarium oxysporum* (NCIM-1282), *Candida albicans* (NCIM-3471), *Aspergillus flavus* (NCIM-539), and *Aspergillus Niger* (NCIM-1196).

The antimicrobial activity of compounds was monitored by observing their minimum inhibitory concentration (MIC, µg/mL) as previously mentioned [28] by broth dilution methods with ciprofloxacin and ampicillin as control drugs. While the antifungal study was carried by the standard agar dilution method, fluconazole, and miconazole used as control drugs.

All the synthesized compounds were also tested for their anticancer activity on mammalian cell lines MCF-7 and BT-474 human breast cancer cell line. This test is performed as previously mentioned MTT colorimetric assay [29]. The cytotoxicity of the synthesized compounds was determined by calculating their IC_{50} values, concentration of compound required to inhibit 50% of cell growth compared with untreated control cells. The IC_{50} values were presented in micromolar per milliliter (µ*M*/mL). Adriamycin was used as positive control for the comparison of cytotoxicity of synthesized compounds. Therefore, our current work is highlighted synthesis, structure activity relationship, and biological evaluation of thiazolone and its derivatives for their anticancer, antibacterial, and antifungal activity.

The compound (**3**) was subjected to a Knoevenagel condensation with the appropriate rhodanine, which had

Scheme 1. Synthesis of (*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl) amino)-substituted acid (**6a**–**l**).

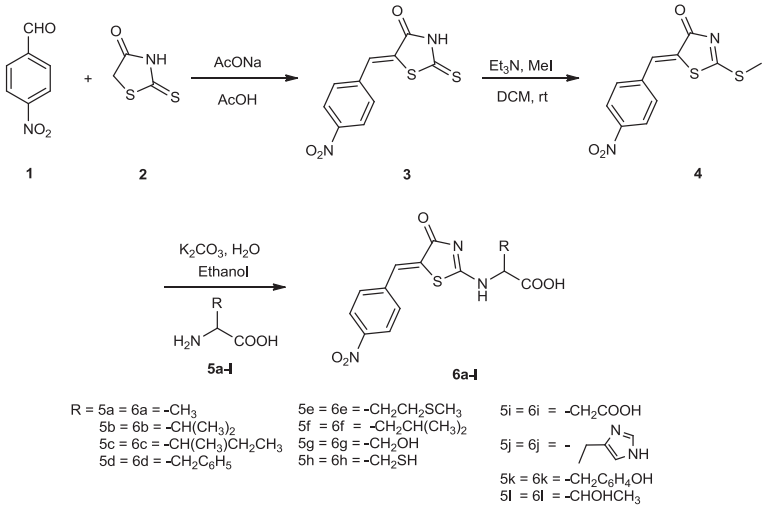
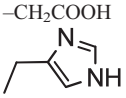


Table 1

Synthesis of (*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-substituted acid (**6a**–**l**)[a].

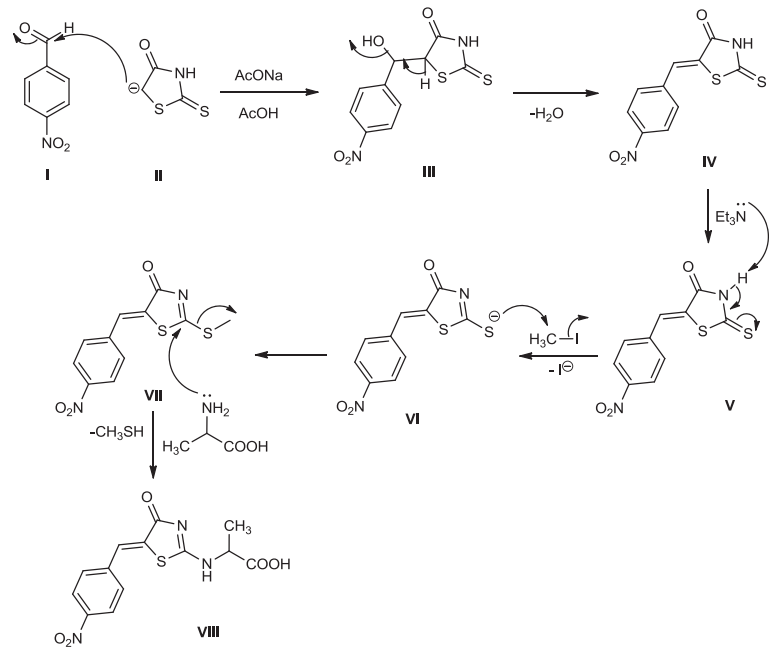
Compound	Substituent (R)	Time (h/min)	Yield[b] (%)	Melting point (°C)
3	—	4 h	90	255–257
4	—	2 h	92	160–162
6a	$-CH_3$	15 min	95	178–180
6b	$-CH(CH_3)_2$	15 min	94	188–190
6c	$-CH(CH_3)CH_2CH_3$	20 min	93	200–202
6d	$-CH_2C_6H_5$	18 min	92	205–207
6e	$-CH_2CH_2SCH_3$	20 min	90	194–196
6f	$-CH_2CH(CH_3)_2$	15 min	92	203–205
6g	$-CH_2OH$	15 min	94	182–184
6h	$-CH_2SH$	15 min	95	190–192
6i	$-CH_2COOH$	20 min	92	204–206
6j		20 min	90	162–164
6k	$-CH_2C_6H_4OH$	15 min	94	218–220
6l	$-CHOHCH_3$	15 min	92	175–177

[a]Reaction condition (**6a**–**l**): compound **(4)** (1 mmol), amino acids (**5a**–**l**) (1.2 mmol), K_2CO_3 (1.2 mmol), and ethanol 1 mL.
[b]Isolated yields.

themselves been synthesized using the reported procedure [30,31]. The structures of the desired compounds were confirmed by IR, ^{1}H NMR, and mass spectral analysis. The compound (*Z*)-5-(4-nitrobenzylidene)-2-thioxothiazolidin-4-one (**3**) was prepared in prominent good yields between the corresponding heterocyclic cores of rhodanine, and 4-nitrobenzaldehyde (Scheme 1). The 2-thioxothiazolidin-4-one-based compounds were synthesized by conventional heating with sodium acetate, which acts as a base and glacial acetic acid as catalysts.

The IR spectrum of compound (**3**) showed a strong absorption band at 1712 cm^{-1} that is due to a carbonyl group. The mass spectrum revealed a molecular ion peak at m/z = 267.40 corresponding to a molecular formula $C_{10}H_6N_2O_3S_2$. The ^{1}H NMR spectra of compounds (**3**) show only one signal for the methyne proton in the range δ 7.70 ppm, at lower field values than those expected for the *E*-isomers, which was strongly indicated that the compounds have the *Z*-configuration. The latter has been reported as thermodynamically more stable than the *E*-configuration [32,33]. The compound (**4**) was synthesized from the compound (**3**), and the structures of the desired compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral analysis. The IR spectrum of (*Z*)-2-(methylthio)-5-(4-nitrobenzylidene) thiazol-4(5H)-one (**4**) showed a strong absorption band at 1698 cm^{-1} that is due to a carbonyl group. The mass spectrum revealed a molecular ion peak at m/z = 280.30 corresponding to a molecular formula $C_{11}H_8N_2O_3S_2$. The ^{1}H NMR spectra of the compounds (**4**) show only one signal for the methyne proton in the range δ 7.80 ppm, and sulfur attached to methyl proton shows the singlet in the range δ 2.80 ppm. We have synthesized the novel series of (*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-substituted acid (**6a**–**l**) from compound (**4**) and different types of *l*-amino acids (**5a**–**l**) (Scheme 1, Table 1). To remove the methylthio group by various *l*-amino acids

Scheme 2. Plausible reaction mechanism for the synthesis of (*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)propanoic acid.



from the C2 position of the thiazole ring and structures of the desired compounds (**6a**–**l**) were confirmed by spectral analysis. The IR spectrum of ((*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) propanoic acid (**6a**) showed a strong absorption band at 1693 cm^{-1} that is due to a carbonyl of carboxylic group and 3467 cm^{-1} due to hydroxyl of carboxylic group. The mass spectrum revealed a molecular ion peak at m/z = 321.31 corresponding to a molecular formula $C_{13}H_{11}N_3O_5S$. Their ^1H NMR spectra revealed the signals of (**6a**) as a representative example, which show one signal for the methyne proton in the range δ 7.40 ppm, phenyl ring proton shows the doublet in the range δ 7.50–7.51 ppm and δ 7.60–7.61 ppm, one of the methyl group proton shows doublet in the range of δ 1.50–1.51 ppm and adjacent to carboxylic acid proton shows quartet in the range of δ 4.50–4.56 ppm, amine group proton shows singlet in the range of δ 9.05 ppm, and carboxylic acid proton shows singlet in the range of δ 11.70 ppm.

The plausible reaction mechanism for the synthesis of (Z)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)propanoic acid is presented in Scheme 2. The first step involves the Knoevenagel condensation of 4-nitrobenzaldehyde (**I**) with rhodanine (**II**) to deliver formation of hydroxyl group (**III**) and then loss of water molecule to form benzylidene derivative (**IV**). After that addition of triethyl amine, it abstract the proton from the nitrogen atom (**V**), resonance occurs, and negative charge comes on sulfur (**VI**) and that was displaced to the iodine from *S*-methylation compound (**VII**). Addition of l-alanine and it was displaced to the thiomethyl group by amine group of the l-alanine to produced final product (**VIII**).

Biological results. All the synthesized compounds (**3**, **4**, and **6a**–**l**) were screened for their in vitro antimicrobial activity against six bacteria, *B. subtilis* (NCIM-2063), *S. aureus* (NCIM-2901), *E. coli* (NCIM-2256), *E. faecalis* (NCIM-5443), *P. aeruginosa* (NCIM-2037),

Table 2

Antibacterial activity of the synthesized compounds **3**, **4**, and **6a**–**l**. (MIC/MBC values (μg/mL)).[a]

Compound		*B.s.*	*S.a.*	*E.c.*	*E.f.*	*P.a.*	*S.t.*
3	MIC	38.70	37.57	41.37	33.70	34.90	32.95
	MBC	57.18	55.19	58.46	47.16	35.74	32.73
4	MIC	36.70	36.50	34.39	34.16	36.33	37.94
	MBC	46.13	46.17	46.40	43.12	56.73	51.75
6a	MIC	35.14	35.12	32.17	33.16	38.35	38.35
	MBC	45.55	45.56	45.53	57.54	47.16	42.18
6b	MIC	6.50	43.10	46.40	48.10	50.13	50.15
	MBC	28.20	53.30	69.40	63.10	52.20	61.90
6c	MIC	52.70	53.57	53.37	31.70	32.90	6.85
	MBC	59.14	59.16	49.40	59.10	39.76	15.85
6d	MIC	32.70	32.50	33.39	33.10	23.30	35.90
	MBC	49.15	49.10	49.40	49.10	59.60	34.70
6e	MIC	9.20	8.25	32.10	32.10	26.50	26.30
	MBC	35.10	32.10	48.50	48.50	32.70	33.10
6f	MIC	8.45	8.78	48.40	48.10	52.13	52.15
	MBC	18.20	20.30	59.40	60.10	55.20	56.90
6g	MIC	37.70	37.57	16.37	38.70	32.90	6.95
	MBC	53.13	55.15	35.40	58.10	37.76	18.75
6h	MIC	33.70	33.50	33.39	35.10	26.30	36.90
	MBC	48.10	47.15	47.47	48.10	58.60	32.70
6i	MIC	9.50	8.10	30.10	34.10	28.50	27.30
	MBC	33.10	34.10	48.50	45.50	35.70	33.10
6j	MIC	7.10	8.50	38.40	38.10	42.13	51.15
	MBC	17.20	20.30	59.40	60.10	55.20	56.90
6k	MIC	36.70	38.57	18.37	39.70	33.90	8.95
	MBC	59.13	54.15	36.40	36.10	36.76	16.75
6l	MIC	37.10	38.10	39.40	36.10	32.13	32.15
	MBC	44.20	42.30	58.40	63.10	55.20	56.90
Ciprofloxacin	MIC	14.70	13.69	12.69	15.69	11.69	15.69
	MBC	33.19	33.10	22.10	32.10	14.10	34.10
Ampicillin	MIC	3.51	3.50	3.50	3.50	3.86	3.46
	MBC	6.29	6.43	2.43	2.43	2.71	2.86

[a]Values are the average of three readings.
B.s., Bacillus subtilis (NCIM-2063); *S.a.*, *Staphylococcus aureus* (NCIM-2901); *E.c.*, *Escherichia coli* (NCIM-2256); *E.f.*, *Enterococcus faecalis* (NCIM-5443); *P.a.*, *Pseudomonas aeruginosa* (NCIM-2037); *S.t.*, *Salmonella typhimurium* (NCIM-2501).

S. typhimurium (NCIM-2501), and six fungal strains, *A. oryzae* (NCIM-570), *P. chrysogenum* (NCIM-707), *F. oxysporum* (NCIM-1282), *C. albicans* (NCIM-3471), *Aspergillus flavus* (NCIM-539), and *Aspergillus Niger* (NCIM-1196), were fluconazole and miconazole used as control drugs. Ethanol was used as solvent control for both antibacterial and antifungal testing (Tables 2 and 3).

All the synthesized compounds were tested for their general cytotoxicity on mammalian cell lines MCF-7, human breast cancer cell line (Table 4). This test is performed as previously mentioned MTT colorimetric assay. The cytotoxicity of the compounds was determined by calculating their IC_{50} values, concentration of compound required to inhibit 50% of cell growth compared with untreated control cells. The IC_{50} values were presented in micromolar per milliliter (μ*M*/mL). The Adriamycin was used as positive control for the comparison of cytotoxicity of synthesized compounds. The assays were performed in triplicate on three independent experiments, and what they mean is taken as a final reading.

The antimicrobial activities of the synthesized compounds against selected Gram-positive and Gram-negative bacteria and multidrug-resistant bacteria are illustrated in Table 2 (Fig. 2) and Table 3 (Fig. 3). The synthesized compounds of present novel series show variety of antibacterial and antifungal activity, ranging from broad spectrum molecule active against the majority of bacterial and fungal strains tested to the narrow spectrum compounds, active only against only one strains. Among the series compounds (**6b**, **6c, 6e, 6f**, **6i**, and **6j**), they are found to be most active molecules, and they are specific toward the gram-positive bacterial, *S. aureus* and *B. subtilis*, *S. typhimurium*. The compound **6b** is more active (MIC of 6.50 μg/mL) against *B. subtilis* and compound **6c** (MIC of 6.85 μg/mL) against *S. typhimurium* than both standards used in the experiment, while the compounds **6i** and **6j** (MIC of 9.50 and 8.10 μg/mL against *B. subtilis* and *S. aureus*) have more activity than ciprofloxacin. Although compounds **6a, 6c, 6e, 6g**, and **6k** have somewhat better activity than

Table 3

Antifungal activity of synthesized compounds **3, 4**, and **6a**–**l** (MIC/MFC values (μg/mL)).[a]

Compound		*A.o.*	*P.c.*	*F.o.*	*C.a.*	*A.f.*	*A.n.*
3	MIC	34.50	54.39	36.50	36.50	19.30	19.30
	MFC	55.10	55.10	75.12	55.10	32.20	72.33
4	MIC	35.00	35.20	35.20	35.20	32.20	32.00
	MFC	39.20	48.60	87.10	46.30	38.88	45.00
6a	MIC	31.50	31.39	11.50	34.50	12.30	19.30
	MFC	48.10	48.10	24.12	36.10	36.20	44.33
6b	MIC	36.00	36.20	35.20	33.20	33.20	34.00
	MFC	43.20	44.50	45.10	66.30	33.88	48.00
6c	MIC	35.50	17.39	17.50	57.50	36.30	39.30
	MFC	49.40	34.40	34.32	36.30	34.30	35.33
6d	MIC	32.30	35.40	32.30	57.50	67.50	34.50
	MFC	42.30	52.20	84.10	52.00	72.30	51.50
6e	MIC	36.30	13.80	51.20	12.25	44.25	30.55
	MFC	36.80	34.70	65.18	20.18	30.64	45.69
6f	MIC	34.00	34.20	34.20	55.20	34.70	31.00
	MFC	43.20	44.60	49.10	65.35	35.88	48.00
6g	MIC	55.55	16.36	39.50	18.50	16.30	36.30
	MFC	60.10	44.10	32.11	35.10	32.22	32.23
6h	MIC	12.00	32.50	34.40	68.40	58.10	34.40
	MFC	51.34	52.25	84.15	72.05	33.30	54.50
6i	MIC	42.35	55.80	41.26	31.26	74.26	35.58
	MFC	55.80	35.78	62.38	62.38	32.84	43.29
6j	MIC	13.40	34.24	54.26	34.66	34.26	33.07
	MFC	23.25	42.65	43.15	64.48	33.89	58.08
6k	MIC	34.55	17.30	16.50	16.50	16.30	33.30
	MFC	45.10	33.75	53.52	32.10	34.20	35.33
6l	MIC	42.00	32.60	62.50	59.50	58.70	34.00
	MFC	58.30	55.20	64.10	62.50	62.30	51.50
Fluconazole	MIC	4.60	2.68	28.65	4.70	8.42	2.68
	MFC	8.35	6.75	46.00	9.62	18.80	4.75
Miconazole	MIC	42.25	6.30	8.18	2.34	49.20	152.30
	MFC	83.18	150.28	18.20	6.18	142.20	142.12

[a]Values are the average of three readings.
A.o., *Aspergillus oryzae* (NCIM-570); *P.c.*, *Penicillium chrysogenum* (NCIM-707); *F.o.*, *Fusarium oxysporum* (NCIM-1282); *C.a.*, *Candida albicans* (NCIM-3471); *A.f.*, *Aspergillus flavus* (NCIM-539); *A.n.*, *Aspergillus Niger* (NCIM-1196).

Table 4

In vitro cytotoxicity of compound toward the MCF-7 and BT-474 cells after 24 h.

SrSr. No.	Compound	$(IC_{50})^a \mu M^b$ MCF-7[c]	BT-474[d]
1	**3**	48.6	63.4
2	**4**	59.8	65.0
3	**6a**	54.9	48.4
4	**6b**	46.2	62.2
5	**6c**	56.3	56.6
6	**6d**	82.4	48.6
7	**6e**	7.5	8.6
8	**6f**	87.4	55.8
9	**6g**	1.4	0.6
10	**6h**	7.2	6.1
11	**6i**	61.3	71.1
12	**6j**	78.7	74.5
13	**6k**	4.1	10.1
14	**6l**	1.6	1.2
	Adriamycin[e]	0.9	0.5

[a]GI_{50}(growth inhibition of 50): concentration of drug that decreases the growth of the cells by 50 compared with non-treated control cell.
[b]Values are the average of three readings.
[c]MCF-7: human breast cancer cell line.
[d]BT-474: human breast cancer cell line.
[e]Adriamycin: positive control compound.

both the standard drugs, what is more important is that they are broad spectrum in nature and they show the activity against majority of bacterial and fungal strains, against bacteria *S. aureus* and *B. subtilis* and fungus *A. oryzae*, *F. oxysporum*, and *C. albicans.*

The compounds **6h** and **6j** are fungal-specific molecule, which is specifically active toward the *A. oryzae*. Remaining compounds of the series **6d, 6f, 6i,** and **6l** have very high MIC values, and therefore, they are inactive as antimicrobial agents.

The newly synthesized 14 compounds were screened for their in vitro growth inhibitory activities against two human breast cancer cells line MCF-7 and BT-474, 100 μ*M*/mL by MTT assays method (Table 4, Fig. 4). The results are shown as percentage cytotoxicity after 24 h.

The compounds found active in preliminary screening were further studied for their cytotoxic effect on human breast cancer cell line MCF-7 and BT-474 cell lines, and the results are expressed as IC_{50}. Among these 14 newly synthesized thiazole derivatives screened for their cytotoxic effect on MCF-7 and BT-474 cells, five compounds showed percentage cell death greater against cell lines used. Among the most active three compounds, two compounds exhibited cell death greater than 50% against both cell lines. The synthesized compounds **6e**, **6g**, **6h**, **6k**, and **6l** showed maximum percentage cytotoxicity 100 μ*M*/mL. The cytotoxicity studies of compounds **6g** and **6l** against MFC-7 and BT-474 cell lines exhibited IC_{50} values are 1.4, 0.6, 1.6, and 1.2 μ*M*/mL, respectively. While the compounds **6e**, **6h**, and **6k** against these two cell lines exhibited IC_{50}: 7.6, 8.5, 7.2, 6.1, 4.1, and 10.1 μ*M*/mL, respectively. The IC_{50} of reference drug adriamycin against MFC-7 and BT-474 cells was found to be 0.9 and 0.5 μ*M*/mL, respectively. The cytotoxicity of all newly synthesized thiazole derivatives mainly depends on type of substitution on thiazole moiety. The substitution pattern of amino acids showed variation in cytotoxicity. The compounds with substituted hydroxyl group attached to thiazole ring that contain amino acids showed the highest percentage of cell death. While the compounds having electron-releasing alkyl chain, methyl-1*H*-imidazole ring group on thiazole rings resulted in loss of activity.

The results of the antimicrobial screening demonstrated some definite and interesting facts about the structural-activity relationship of synthesized thiazole moiety. In majority of cases, dependence of activity profile on structural modifications of the molecule is clear and fascinating. Because of different types of amino acid and

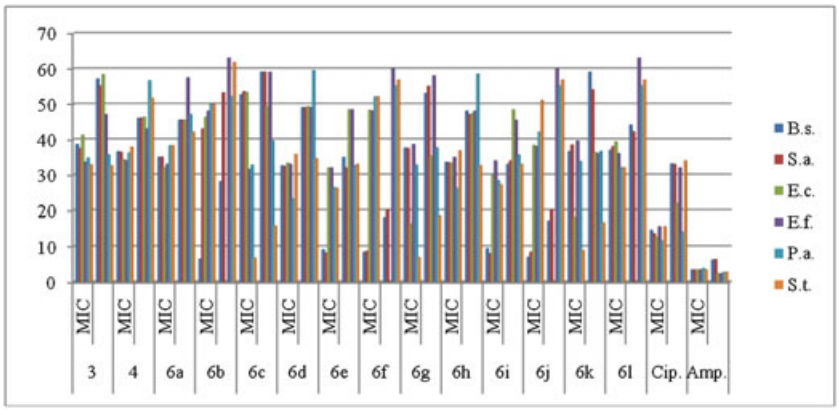


Figure 2. Antibacterial activities of the synthesized compounds **3**, **4** and **6a**–**l** (MIC/MBC values (μg/mL)). [Color figure can be viewed at wileyonlinelibrary.com]

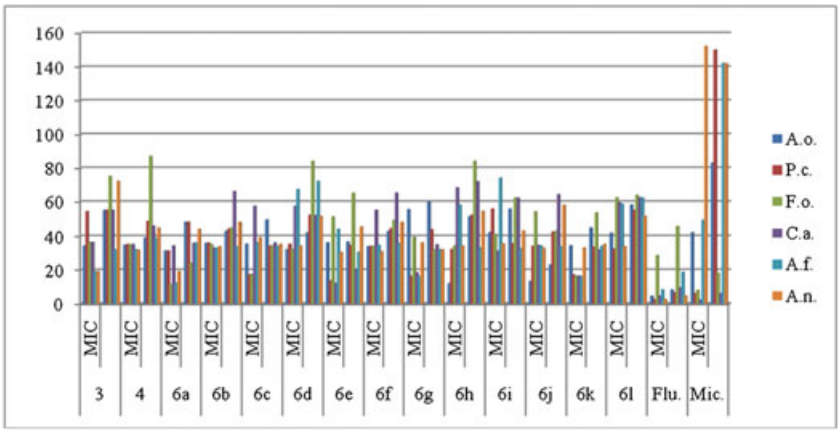


Figure 3. Antifungal activities of synthesized compounds **3**, **4**, and **6a**–**l** (MIC/MFC values (μg/mL)). [Color figure can be viewed at wileyonlinelibrary.com]

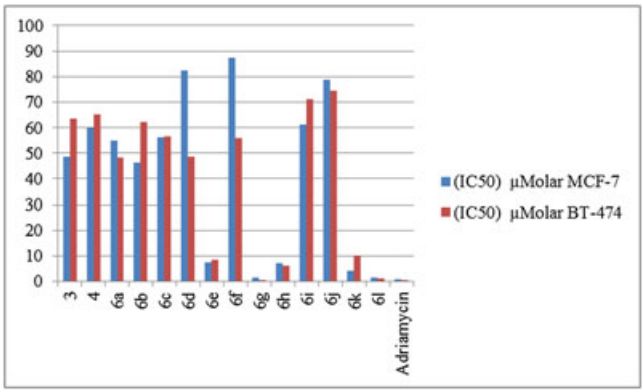


Figure 4. In vitro cytotoxicity of compound toward the MCF-7 and BT-474 cells after 24 h. [Color figure can be viewed at wileyonlinelibrary.com]

variation in activity profile of molecules, they are also directly attributed with the structural variations. The important highlights of structure–activity relationship are as follows.

Effect of alkane chain. In the present study, it is clear that the activity profile of molecule is strongly affected by the branching pattern and chain length of alkanes chain. Attachment of methyl group at C2 position on the thiazole moiety (**6a**) makes molecule active against bacterial strains may be due to its small size and electron-donating effects. When this methyl group is replaced by 2-methylbutyl (**6c**), the molecules become specific and active against selected of strains. This clearly shows that branching at this point has a positive effect for antimicrobial activity of molecule. However, attachment of 2-methylpropyl (**6f**) at the same position make the molecule specific toward the *B. subtilis*.

Effect of alkane chain with hydroxyl group. The compounds **6g** and **6l** containing alkane chain with hydroxyl groups with different position. Substitution by hydroxymethyl **(6g**) at C2 position on the thiazolone moiet**y** makes the molecule selective active against bacterial and fungal strains.

Effect of phenyl ring. Substitution by phenylmethyl (**6d**) at C2 position on the thiazolone moiety gives the inactive against all bacterial and fungal strains, while 4-hydroxyphenyl (**6k**) substitution makes the molecule active. This observation clearly shows that only phenyl ring gives the slightly inactive molecule, and addition of hydroxyl group at C4 position causes better in this activity.

Effect of heterocyclic ring. The compound **6j** containing methyl-imidazole ring at C2 position on the thiazole moiety makes the molecule specifically active toward Gram-positive bacteria *B. subtilis*, *S. aureus*, and *A. oryzae*.

Effect of sulfur containing group. The compound (**6e**) with terminal methylthio group is only fungal specific and active against *P. chrysogenum* and *C. albicans*, while compound **6h** with terminal mercapto group shows specificity toward Gram-positive bacteria *A. oryzae.*

Highlight of synthesis of novel (*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-substituted acid (6a–l).
The novelty and highlight in synthesis of (*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-substituted acid are (i) the first reported synthesis, using ethanol and water as solvent; (ii) development of eco-friendly process by omission of organic solvents, (iii) evasion of cumbersome workup procedures; (iv) ethanol and water, which are non-toxic and economically feasible to use this reactions; (v) excellent yields in shorter reaction time making the process economically lucrative for industrial application; (vi) opening the horizon for the synthesis of series novel compounds (**3**, **4**, and **6a–l**) with promising anticancer, antibacterial, and antifungal activity, which are yet to be explored and exploring them for other biological applications.

CONCLUSIONS

The newly synthesized 14 compounds are reported at room temperature, less reaction time with good to excellent yields. All the synthesized compounds were also tested for their in vitro anticancer activity against MCF-7 and BT-474 human breast cancer cell lines. Our present study is to synthesize and investigate the potent anticancer, antibacterial, and antifungal activities of some new (*Z*)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-substituted acid with the hope of discovering new structures that could be used as potent antimicrobial agents. Among these, the compounds **6a**, **6b,** **6c**, **6e**, **6f**, **6g**, **6h**, **6i**, **6j**, and **6k** showed highest antibacterial and antifungal activity among the series. The compound **6a** exhibited significant antibacterial activity against *B. subtilis*, whereas compound **6j** displayed significant antifungal activity against fungal strains, that is, *A. oryzae*. The in vitro anticancer studies revealed that **6e**, **6g**, **6h**, **6k**, and **6l** are the most active compounds against MCF-7 and BT-474 human breast cancer cell lines, which can be regarded as the promising drug candidate for development of anticancer drugs. Our aim has been verified by the synthesis of thiazole moiety and attached to C2 position different types of l-amino acids, which are very effective for the enhanced anticancer and antimicrobial activities.

EXPERIMENTAL

Material and methods. 2-Thioxothiazolidin-4-one (rhodanine), 4-nitrobenzaldehyde, anhydrous sodium acetate, triethylamine, amino acids, dichloromethane, iodomethane, and various solvents were commercially available. The major chemicals were purchased from Sigma Aldrich and Avra labs. Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates. Developed plates were examined with UV lamps (254 nm). IR spectra were recorded on an FTIR (Bruker). Melting points were recorded on SRS Optimelt, melting point apparatus, and are uncorrected. ^{1}H NMR spectra were recorded on a 400-MHz Bruker spectrometer. Chemical shifts are reported as δ_{ppm} units. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

General procedure for the synthesis of compounds (3).
In a 100-mL round bottom flask, the equimolar amount of 2-thioxothiazolidin-4-one (1 mmol), anhydrous sodium acetate (1 mmol), glacial acetic acid (1 mL), and 4-nitrobenzaldehyde were added to the reaction mixture. The mixture was stirred under reflux condition for 1 h. The progress of reaction was monitored by TLC (20% ethyl acetate: *n*-hexane). After completion of the reaction, the reaction mixture was poured into the ice-cold water. The precipitate was filtered, washed with water (3 × 10 mL), dried, and purified by recrystalization in ethanol as solvent to give 90% yield.

Yellow solid, yield: 90%. mp 255–257°C; ES-MS *m*/*z* (%): 267.40. IR νmax/cm^{-1}: 3265 (NH), 1712 (C═O), 1606 (C═C), 1510 (C═N), 1286 (C═S), 1184 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 7.70 (s, 1H, ═CH), 7.86–7.87 (d, 2H, Ar–CH), 8.35–8.46 (d, 2H, Ar–CH), 14.00 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 116.5, 123.5, 129.3, 141.4, 143.4, 147.2, 168.5, 193.7.

General procedure for the synthesis of compounds (4).
In a 100-mL round bottom flask, the compound **(3)** (1 mmol), triethylamine (1.5 mmol), was added in dichloromethane (1 mL) at room temperature. To the stirred reaction mixture with iodomethane (1.5 mmol) was added and stirred for 30 min at room temperature. The progress of reaction was monitored by TLC (10% chloroform: methanol). After completion of reaction, the reaction mixture was concentrated in vacuo. The residue was washed out with water (3 × 15 mL) to afford the crude product. The crude product was recrystallized using ethanol as solvent to give yield in the range 92%.

Yellow solid, yield: 92%. mp 160–162°C; ES-MS *m*/*z* (%): 280.30. IR νmax/cm^{-1}: 3041 (CH–Ar), 1696 (C═O), 1591 (C═C), 1518 (C═N), 1295 (C═S), 1086 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 2.80 (s, 3H, CH_3), 7.80 (s, 1H, ═CH), 7.85–7.86 (d, 2H, Ar–CH), 8.25–8.26 (d, 2H, Ar–CH), ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 14.5, 123.9, 129.3, 132.5, 141.4, 147.4, 152.3, 162.2, 168.5.

General procedure for the synthesis of (*Z*)-2-((5-(4-nitro benzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-substituted acids (6a–l).. In a 100-mL round bottom flask, the compound **(4)** (1 mmol), amino acids (1.5 mmol),

potassium carbonate (K_2CO_3) (1.5 mmol), ethanol (1 mL), and water (1 mL) were added, and this mixture was stirred for 15–20 min at room temperature. The progress of reaction was monitored by TLC (10% chloroform: methanol). After completion of reaction, the reaction mixture was concentrated in vacuo. The residue was washed with water (3 × 15 mL) to afford the crude product. The compounds (**6a**–**l**) were recrystallized from ethanol and isolated as yellowish powders.

(Z)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl) amino)propanoic acid (6a). Yellow solid, ES-MS *m*/*z* (%): 321.31. IR νmax/cm^{-1}: 3467 (OH), 3277 (NH), 2377 (NO_2), 1729 (HO–C═O), 1693 (C═O), 1564 (C═C), 1513 (C═N), 1174 (C–S), 844 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.50–1.51 (d, 3H, C–CH_3), 4.50–4.56 (q, 1H, CH), 7.40 (s, 1H, ═CH), 7.50–7.51 (d, 2H, Ar–CH), 7.60–7.61 (d, 2H, Ar–CH), 9.05 (s, 1H, NH), 11.70 (s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 16.7, 53.2, 123.9, 129.3, 132.7, 141.5, 147.1, 152.3, 158.3, 167.7, 174.2.

(Z)-3-methyl-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) butanoic acid (6b). Yellow solid, ES-MS *m*/*z* (%): 349.36. IR νmax/cm^{-1}: 3419 (OH), 3210 (NH), 3013 (CH–Ar), 1730 (HO–C═O), 1689 (C═O), 1551 (C═C), 1593 (C═N), 1012 (C–S), 1091 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 0.91–0.98 (d, 6H, $CH(CH_3)_2$), 1.50–1.58 (m, 1H, CH), 4.40–4.41 (d, 1H, CH), 7.88 (s, 1H, ═CH), 7.91–7.92 (d, 2H, Ar–CH), 8.33–8.34 (d, 2H, Ar–CH), 11.71 (s, 1H, NH), 11.12 (s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 18.9, 30.2, 61.2, 123.2, 129.2, 132.3, 141.9, 147.3, 152.1, 158.7, 167.2, 174.2.

(Z)-3-methyl-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)pentanoic acid (6c). Yellow solid, ES-MS *m*/*z* (%): 363.39. IR νmax/cm^{-1}: 3398 (OH), 3212 (NH), 3027 (CH–Ar), 1725 (HO–C═O), 1692 (C═O), 1557 (C═C), 1583 (C═N), 1013 (C–S), 1097 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 0.91–0.98 (t, 3H, CH_2–CH_3), 1.19–1.20 (d, 3H, CH_3) 1.52–1.60 (m, 2H, CH_2), 1.81–1.89 (m, 1H, CH), 4.43–4.44 (d, 1H, CH), 7.70 (s, 1H, ═CH), 7.90–7.91 (d, 2H, Ar–CH), 8.25–8.26 (d, 2H, Ar–CH), 11.62 (s, 1H, NH), 13.11 (s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 11.3, 15.5, 25.6, 37.3, 58.4, 113.4, 124.9, 129.6, 132.3, 141.9, 152.9, 158.1, 167.3, 174.2.

(Z)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl) amino)-3-phenyl propanoic acid (6d). Yellow solid, ES-MS *m*/*z* (%): 397.40. IR νmax/cm^{-1}: 3436 (OH), 3214 (NH), 2992 (CH–Ar), 1727 (HO–C═O), 1695 (C═O), 1551 (C═C), 1581 (C═N), 1017 (C–S), 1098 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 2.50–2.51 (d, 2H, CH_2), 4.43–4.51 (q, 1H, CH), 7.80 (s, 1H, ═CH), 7.92–7.93 (d, 2H, Ar–CH), 8.32–8.33 (d, 2H, Ar–CH), 11.75 (s, 1H, NH), 13.15 (s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 36.4, 58.4, 124.9, 125.9, 125.7, 128.6, 128.9, 130.7, 132.3, 135.3, 136.9, 152.2, 158.5, 167.1, 175.2.

(Z)-4-(methylthio)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)butanoic acid (6e). Yellow solid, ES-MS *m*/*z* (%): 381.43. IR νmax/cm^{-1}: 3398 (OH), 3210 (NH), 2981 (CH–Ar), 1723 (HO–C═O), 1697 (C═O), 1541 (C═C), 1586 (C═N), 1014 (C–S), 1091 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 2.40–2.45 (q, 2H, CH_2), 2.48–2.52 (t, 2H, CH_2), 3.80 (s, 3H, CH_3), 4.43–4.48 (t, 1H, CH), 7.78 (s, 1H, ═CH), 7.82–7.83 (d, 2H, Ar–CH), 7.98–7.99 (d, 2H, Ar–CH), 8.42 (s, 1H, NH), 8.62 (s, 1H, COOH).^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 15.3, 29.8, 30.4, 56.9, 124.9, 127.7, 128.6, 136.9, 152.2, 158.5, 162.4, 167.3, 174.7.

(Z)-4-methyl-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)pentanoic acid (6f). Yellow solid, ES-MS *m*/*z* (%): 363.39. IR νmax/cm^{-1}: 3397 (OH), 3212 (NH), 3013 (CH–Ar), 1734 (HO–C═O), 1691 (C═O), 1555 (C═C), 1583 (C═N), 1013 (C–S), 1091 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 0.90–0.91 (d, 6H, CH–$(CH_3)_2$), 1.41–1.48 (m, 1H, CH), 1.71–1.75 (t, 2H, CH_2), 4.44–4.49 (q, 1H, CH), 7.76 (s, 1H, ═CH), 7.85–7.86 (d, 2H, Ar–CH), 7.96–7.97 (d, 2H, Ar–CH), 11.72 (s, 1H, NH), 13.14 (s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 22.7, 24.4, 40.3, 55.4, 113.4, 124.9, 129.2, 132.1, 152.2, 159.1, 162.3, 167.1, 174.2.

(Z)-3-hydroxy-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)propanoic acid (6g). Yellow solid, ES-MS *m*/*z* (%): 337.31. IR νmax/cm^{-1}: 3410 (OH), 3210 (NH), 3018 (CH–Ar), 1729 (HO–C═O), 1690 (C═O), 1551 (C═C), 1511 (C═N), 1019 (C–S), 1097 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 3.62 (s, 1H, OH), 4.02–4.19 (t, 1H, CH), 4.23–4.24 (d, 2H, CH_2), 7.77 (s, 1H, ═CH), 7.81–7.82 (d, 2H, Ar–CH), 8.14–8.15 (d, 2H, Ar–CH), 11.60 (s, 1H, NH), 13.13 (s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 59.2, 62.2, 124.9, 129.2, 130.3, 132.1, 136.4, 151.9, 158.1, 167.9, 172.2.

(Z)-3-mercapto-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)propanoic acid (6h). Yellow, ES-MS *m*/*z* (%): 353.37. IR νmax/cm^{-1}: 3415 (OH), 3208 (NH), 3017 (CH–Ar), 2500 (SH), 1728 (HO–C═O), 1695 (C═O), 1559 (C═C), 1501 (C═N), 1011 (C–S), 1099 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.51 (s, 1H, SH), 3.11–3.12 (d, 2H, CH_2), 4.13–4.17 (t, 1H, CH), 7.81 (s, 1H, ═CH), 7.98–7.99 (d, 2H, Ar–CH), 8.15–8.16 (d, 2H, Ar–CH),11.61 (s, 1H, NH), 13.11 (s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 26.3, 60.4, 124.9, 127.5, 132.5, 135.6, 152.9, 158.3, 162.3, 167.2, 178.2.

(Z)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl) amino)succinic acid (6i). Yellow solid, ES-MS *m*/*z* (%): 365.32. IR νmax/cm^{-1}: 3424 (OH), 3213 (NH), 3020 (CH–Ar), 1722 (HO–C═O), 1692 (C═O), 1549 (C═C), 1503 (C═N), 1030 (C–S), 1080 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 2.60–2.61 (d, 2H, CH_2), 3.71–3.78 (t, 1H, CH), 7.78 (s, 1H, ═CH), 7.98–7.99 (d, 2H, Ar–CH), 8.21–8.22 (d, 2H, Ar–CH), 11.70 (s, 1H, NH), 13.12 (s, 2H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 35.9, 53.3, 124.9, 130.1, 132.5, 135.6, 136.4, 152.9, 158.3, 162.3, 167.2, 178.2.

(Z)-3-(1H-imidazol-4-yl)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)propanoic acid (6j). Yellow solid, ES-MS *m/z* (%): 387.64. IR νmax/cm^{-1}: 3415 (OH), 3210 (NH), 3011 (CH–Ar), 1729 (HO–C═O), 1691 (C═O), 1552 (C═C), 1508 (C═N), 1033 (C–S), 1092 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 2.92–3.20 (d, 2H, CH_2), 3.70–3.85 (t, 1H, CH), 7.77 (s, 1H, ═CH), 7.86 (s, 1H, ═CH), 7.86–7.87 (d, 2H, Ar–CH), 8.10–8.11 (d, 2H, Ar–CH), 8.98 (s, 1H, ═CH), 11.62–(s, 1H, NH), 13.12 (s, 1H, NH), 13.14 (s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 28.9, 58.3, 117.9, 124.9, 124.7, 124.7, 127.9, 128.6, 132.1, 136.2, 152.3, 158.2, 167.5, 176.2.

(Z)-3-(4-hydroxyphenyl)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)propanoic acid (6k). Yellow solid, ES-MS *m/z* (%): 413.40. IR νmax/cm^{-1}: 3456 (O═C–OH), 3397 (OH), 3215 (NH), 2991 (CH–Ar), 1738 (HO–C═O), 1697 (C═O), 1553 (C═C), 1591 (C═N), 1011 (C–S), 1089 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 2.80–2.98 (d, 2H, CH_2), 4.43–4.72 (t, 1H, CH), 5.30 (s, 1H, OH), 7.10–7.11 (d, 2H, Ar–CH), 7.22–7.23 (d, 2H, Ar–CH), 7.72 (s, 1H, ═CH), 7.82–7.83 (d, 2H, Ar–CH), 8.14–8.15 (d, 2H, Ar–CH), 11.62 (s, 1H, NH), 13.11 (s, 1H, COOH).^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 36.3, 58.6, 115.8, 127.7, 128.9, 129.2, 130.2, 135.3, 136.9, 152.2, 155.7, 158.5, 162.7, 167.8, 174.3.

(Z)-3-hydroxy-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)butanoic acid (6l). Yellow solid, ES-MS *m/z* (%): 351.33. IR νmax/cm^{-1}: 3432 (OH), 3203 (NH), 3011 (CH–Ar), 1727 (HO–C═O), 1693 (C═O), 1554 (C═C), 1596 (C═N), 1042 (C–S), 1115 (C–N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.10–1.21 (d, 3H, CH_3), 3.51–3.58 (d, 1H, CH), 3.63 (s, 1H, OH), 3.93–4.01 (m, 1H, CH), 7.76 (s, 1H, ═CH), 7.92–7.93 (d, 2H, Ar–CH), 8.25–8.26 (d, 2H, Ar–CH),11.71 (s, 1H, NH), 13.10 (s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 19.6, 64.3, 66.5, 124.8, 130.3, 132.4, 136.1, 152.2, 158.3, 162.4, 167.8, 174.2.

Acknowledgments. The authors are thankful to the head of Department of Chemical Technology, Dr. Babasaheb Ambedkar, Marathwada University, Aurangabad 431004 (MS), India, for providing the laboratory facility. The authors are also thankful to the Advanced Centre for Treatment Research and Education in Cancer (ACTREC) Mumbai, for anticancer activity.

REFERENCES AND NOTES

[1] Chopra, I.; Hodgson, J.; Metcalf, B.; Poste, G. Antimicrob Agents Chemother 1997, 41, 497.

[2] Kunzler, A.; Neuenfeldt, P. D.; das Neves, A. M.; Pereira, C. M. P.; Marques, G. H.; Nascente, P. S.; Fernandes, M. H. V.; Hubner, S. O.; Cunico, W. Eur J Med Chem 2013, 64, 74.

[3] Nikhil, D.; Amnerkar, M.; Kishore, P.; Bhusari, P. Dig J Nanomater Biostruct 2010, 5, 177.

[4] Hoaglad, H. C. Semin Oncol 1982, 9, 95.

[5] Porter, P. Engl N J Med 2008, 358, 213.

[6] Ramachandran, R.; Rani, M.; Kabilan, S. Bioorg Med Chem Lett 2009, 19, 2819.

[7] El-Gaby, M. S. A.; El-Hag Ali, G. A. M.; El-Maghraby, A. A.; Abd El-Rahman, M. T.; Helal, M. H. M. Eur J Med Chem 2009, 44, 4148.

[8] Tomasic, T.; Zidar, N.; Rupnik, V.; Kovac, A.; Blanot, D.; Gobec, S.; Kikelj, D.; Masic, L. P. Bioorg Med Chem Lett 2009, 19, 153.

[9] Sink, R.; Kovac, A.; Tomasic, T.; Rupnik, V.; Boniface, A.; Bostock, J.; Chopra, I.; Blanot, D.; Masic, L. P.; Gobec, S.; Zega, A. Chem Med Chem 2008, 3, 1362.

[10] Patel, D.; Kumari, P.; Patel, N. Eur J Med Chem 2012, 48, 354.

[11] Sortino, M.; Delgado, P.; Juarez, S.; Quiroqa, J.; Abonia, R.; Insuasty, B.; Noqueras, M.; Rodero, L.; Garibatto, F. M.; Enriz, R. D.; Zacchino, S. A. Bioorg Med Chem 2007, 15, 484.

[12] Ravi, S.; Chiruvella, K. K.; Rajesh, K.; Prabhu, V.; Raghavan, S. C. Eur J Med Chem 2010, 45, 2748.

[13] Havrylyuk, D.; Mosula, L.; Zimenkovsky, B.; Vasylenko, O.; Gzella, A.; Lesyk, R. Eur J Med Chem 2010, 45, 5012.

[14] Sala, M.; Chimento, A.; Saturnino, C.; Gomez-Monterrey, I. M.; Musella, S.; Bertamino, A.; Milite, C.; Sinicropi, M. S.; Caruso, A.; Sirianni, R.; Tortorella, P.; Novellino, E.; Campiglia, P.; Pezzi, V. Bioorg Med Chem Lett 2013, 23, 4990.

[15] Dayam, R.; Sanchez, T.; Clement, O.; Shoemaker, R.; Sei, S.; Neamati, N. J Med Chem 2005, 48, 111.

[16] Ustundag, G. C.; Gursoy, E.; Naesens, L.; Guzeldemirci, N. U.; Capan, G. Bioorg Med Chem 2016, 24, 240.

[17] Li, C.; Liu, J. C.; Li, Y. R.; Gou, C.; Zhang, M. L.; Liu, H. Y.; Li, X. Z.; Zheng, C. J.; Piao, H. R. BioorgMed Chem Lett 2015, 25, 3052.

[18] Manvar, D.; Kuçukguzel, I.; Erensoy, G.; Tatar, E.; Deryabaşogullari, G.; Reddy, H.; Talele, T. T.; Cevik, O.; Kaushik-Basu, N. Biochem Biophys Res Commun 2016, 469, 743.

[19] Kucukguzel, S. G.; Kocatepe, A.; De Clercq, E.; Sahin, F.; Gulluce, M. Eur J Med Chem 2006, 41, 353.

[20] Chandrappa, S.; Kavitha, C. V.; Shahabuddin, M. S.; Vinaya, K.; Ananda Kumar, C. S.; Ranganatha, S. R.; Raghavan, S. C.; Rangappa, K. S. Bioorg Med Chem 2009, 17, 2576.

[21] Balzarini, J.; Orzeszko, B.; Maurin, J. K.; Orzeszko, A. Eur J Med Chem 2007, 42, 993.

[22] Galal, S. A.; Hegab, K. H.; Kassab, A. S.; Rodriguez, M. L.; Kerwin, S. M.; El-Khamry, A. A.; El Diwani, H. I. Eur J Med Chem 2009, 44, 1500.

[23] Asmaa, A.; Magd-El, D.; Amira, S.; Abd-El, A.; Hanaa, M. R.; Mashalla, M. S. J Am Sci 2010, 6, 251.

[24] Dmytro, H.; Ludmyla, M.; Zimenkovsky, B.; Vasylenko, O.; Gzella, A.; Lesyk, R. Eur J Med Chem 2010, 45, 5012.

[25] Bronson, J. J.; Den Bleyker, K. L.; Falk, P. J.; Mate, R. A.; Ho, H. T.; Pucci, M. J.; Snyder, L. B. Bioorg Med Chem Lett 2003, 13, 873.

[26] (a) Pansare, D. N.; Mulla, N. A.; Pawar, C. D.; Shende, V. R.; Shinde, D. B. Bioorg Med Chem Lett 2014, 24, 3569; (b) Darandale, S. N.; Pansare, D. N.; Mulla, N. A.; Shinde, D. B. Bioorg Med Chem Lett 2013, 23, 2632; (c) Darandale, S. N.; Mulla, N. A.; Pansare, D. N.; Sangshetti, J. N.; Shinde, D. B. Eur J Med Chem 2013, 65, 527.

[27] (a) Pansare, D. N.; Shinde, D. B. Tetrahedron Lett 2014, 55, 1107; (b) Pansare, D. N.; Shinde, D. B. Open Chem J 2015, 2, 40; (c) Pansare, D. N.; Shinde, D. B. Res Rev: J Chem 2015, 4, 8; (d) doi.org/10.1016/j.jscs.2015.10.005

[28] Jin, X.; Zheng, C. J.; Song, M. X.; Wu, Y.; Sun, L. P.; Li, Y. J.; Yu, L. J.; Piao, H. R. Eur J Med Chem 2012, 56, 203.

[29] Denizot, F.; Lang, R. J Immunol Methods 1986, 89, 271.

[30] Lesyk, R.; Zimenkovsky, B.; Subtelna, I.; Nektegayev, I.; Kazmirchuk, G. Acta Pol Pharm Drug Res 2003, 6, 457.

[31] Lesyk, R. B.; Zimenkovsky, B. S. Curr Org Chem 2004, 8, 1547.

[32] Ishida, T.; In, Y.; Inoue, M.; Ueno, Y.; Tanaka, C.; Hamanaka, N. Tetrahedron Lett 1989, 30, 959.

[33] Momose, Y.; Meguro, K.; Ikeda, H.; Hatanaka, C.; Oi, S.; Sohda, T. Chem Pharm Bull 1991, 39, 1440.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.